Materials Science and Engineering C 80 (2017) 352–361

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Modification of calcium phosphate cement with poly (γ-glutamic acid) and its strontium salt for kyphoplasty application

Chunxia Gao [a], Huiling Liu [a], Zong-Ping Luo [a,b], Sajilafu [a,b], Huilin Yang [a,b], Lei Yang [a,b,*]

[a] Orthopaedic Institute, Department of Orthopaedics, The First Affiliated Hospital, Soochow University, Suzhou, Jiangsu 215006, PR China
[b] International Research Center for Translational Orthopaedics (IRCTO), Soochow University, Suzhou, Jiangsu 215006, PR China

ARTICLE INFO

Article history:
Received 22 March 2017
Received in revised form 2 May 2017
Accepted 13 May 2017
Available online 14 May 2017

Keywords:
Calcium phosphate cement
Kyphoplasty
Poly (γ-glutamic acid)
Mechanical property
Strontium

ABSTRACT

To meet the requirements of minimally invasive surgical treatments for osteoporotic vertebral compression fracture, various strategies have been proposed to enhance the properties of calcium phosphate cement (CPC). Here, a new strategy was developed by incorporating poly (γ-glutamic acid) (γ-PGA) and its strontium salt into the formulation of alpha tricalcium phosphate (α-TCP)-based CPC. Effects of γ-PGA on the injectability, cohesion, setting times, mechanical compressive strengths and cytocompatibility were systematically studied. Results showed that the injectability, cohesion and setting times were considerably improved by introducing γ-PGA into the CPC. Moreover, after setting for 7 days, the compressive strengths increased from 14.6 ± 1.4 MPa for pure CPC to 35.3 ± 1.7 MPa for CPC with 8.9 wt% γ-PGA and further to 61.2 ± 5.4 MPa for CPC with 8.9 wt% γ-PGA combined with its strontium salt. *In vitro* osteoblast proliferation tests showed that the group of CPC modified by γ-PGA and its strontium salt had better cytocompatibility than the pure CPC group. All results suggest that optimal properties were obtained for the cement with 8.9 wt% γ-PGA added to its solid phases and using strontium salt as the reactive liquid phase, making it as a promising candidate for application in kyphoplasty.

1. Introduction

Osteoporotic vertebral fractures are becoming a major health concern for many nations because of aging populations worldwide. Over the past decade, percutaneous kyphoplasty and vertebraplasty are one of the best minimally invasive options for patients that suffer from osteoporotic vertebral fractures [1,2]. In the procedure of kyphoplasty or vertebraplasty, poly (methyl methacrylate) (PMMA) bone cement is injected into the fractured site for the stabilization and augmentation of the vertebral body. However, the polymerization process of PMMA carries risks of localized thermal tissue necrosis and may cause continued vertebral collapse due to the modulus mismatch with adjacent bones [3]. As a promising candidate, calcium phosphate cement (CPC) has received much attentions in this area due to their excellent biodegradability, bioactivity and osteoconductivity [4].

Although CPCs have shown great potential as augmentation material and some products of CPCs have already been commercialized for bone filler applications [5,6], it is generally accepted that there are still crucial problems that cannot satisfy the clinical requirements of kyphoplasty [7]. Specifically, the pure CPC is generally prone to phase separation during injection which may cause extravasation from the surgical site and detrimental effects on the final properties of the set CPC [8]. Meanwhile, pure CPC tends to decay or disintegrate upon early contact with biological fluids and show fatiguing features because the cement does not own comparable toughness compared to bone [9,10]. In addition to the poor handling properties, another shortcoming of CPCs is their relatively low compressive strengths and susceptibility to brittle fracture, which limits their applications to only the treatment of non-load bearing bone defects [11,12]. To overcome these shortcomings, incorporating various organic additives into the formulations of CPCs has attracted much attention as it has been proved as an effective strategy to reinforce the handling performances of CPCs. However, in this strategy, setting times of CPCs are generally prolonged because the diffusion of calcium and phosphate ions becomes slow due to high viscosity of the liquid phase in CPCs. Our recent study demonstrated that adding natural biomacromolecules including starch and poly (gamma-glutamic acid) is an effective method to improve the injectability, mechanical properties and anti-collapsibility of α-TCP-based CPCs [13,14]. In addition, it has been reported that CPCs containing carboxyl groups from polymers or organic compounds harden rapidly, with an insoluble matrix by chelation. The compressive strengths of CPCs are also affected by the degree of chelation at the early stages of its cementation [15–17]. Although the importance of the chelation in improving the handling performances and mechanical properties has been reported, most studies focus on using citric acid, which has three carboxyl groups and one hydroxyl group, as an additive to CPCs [18–20].

* Corresponding author at: Room 313 Building #1, South Campus of Soochow University, 708 Renmin Road, Suzhou, Jiangsu 215006, PR China.
E-mail address: leiy@suda.edu.cn (L. Yang).

http://dx.doi.org/10.1016/j.msec.2017.05.070
0928-4931/

To further confirm the role of chelation on the improved properties of CPCs, an anionic polypeptide poly (γ-glutamic acid) (γ-PGA) was employed in the present study as an additive because it contains lots of functional carboxyl groups. γ-PGA is a poly(amino acid) formed by the amide bond linkage between the amino groups on the α-carbon and the carboxyl groups on the γ-carbon. Furthermore, it has reactive carboxyl groups on its side chains which allow chemical modification and covalent cross-linking [21,22]. In addition, the salt forms of Na^+, Ca^{2+}, Sr^{2+} and NH_4^+ of γ-PGA are known to be water soluble due to the deprotonation of COOH groups to COO^- groups [23,24]. Therefore, one can hypothesize that the γ-PGA will not only provide functional groups for chelation, but can also introduce various trace elements into the CPCs, due to the special binding characteristics between the carboxyl groups and cations.

Recently strontium (Sr) has received considerable attention due to its dual functions in increasing the bone formation and decreasing the bone resorption [12]. To date, various Sr-doped CPCs have been developed for treating osteoporotic bone fractures [25–27]. C Kasperk et al. showed faster osseointegration and more new bone formation for Sr-composited CPC when compared to CPC in implant sites [27]. Moreover, Sr has positive effect on improving compressive strengths of CPCs. M Gelinsky et al. proved that initial compressive strength can be improved from 8.9 MPa of CPC to 22.9 MPa of Sr modified CPC within 6 h, and further increased up to 31.6 MPa of CPC and 53.3 MPa of Sr modified CPC after aging for 7 days, respectively [25]. Here, $SrCO_3$ was employed as the strontium source to introduce Sr ions into cement.

A main objective of this study is to develop a new injectable CPC formula which has not only good injectability, cohesion and setting properties but also greatly improved compressive strength. In view of all the aims above, γ-PGA and its strontium salt were incorporated into α-TCP-based CPC and the effects of γ-PGA and Sr on the handling properties (*e.g.* injectability, cohesion and setting times), mechanical, and biocompatibility properties and degradability of the modified CPCs were systemically investigated.

2. Materials and methods

2.1. Materials

α-TCP was purchased from Ensail Co., Ltd. (Beijing, China). Calcium hydrogen phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Aldrich (St. Louis, USA). Strontium carbonate ($SrCO_3$, 99%) was purchase from Alfa Aesar (Tianjin, China). Poly (γ-glutamic acid) (γ-PGA, Mw = 1500–2500 kDa) was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ was used as precursors to synthesize hydroxyapatite (HA) in house by the co-precipitation method reported by others [28,29]. All reagents were of analytic purity and used directly without further purification.

2.2. Cement preparation

In this study, three groups of cement were prepared and the formulae were shown in Table 1, including α-TCP-based CPC (named as CPC), γ-PGA-modified CPC (γ-PGA-CPC) and Sr-containing γ-PGA modified CPC (Sr-γ-PGA-CPC). The compositions of these cement were selected based on our previous study [14]. Our previous results demonstrated that the addition of HA could improve the compressive strengths and shorten the setting times of cement. Thus, HA powder was chosen as seed crystals in anticipation to improve the mechanical properties and adjust the setting times of cement.

To make the solid phase of cement, α-TCP, DCPD and γ-PGA were mixed with a predetermined weight ratio and ball-milled in ethanol (powders: agate ball: ethanol = 4:30:9 in weight) at 464 rpm on a planetary ball miller (PM2 L, DPLIFT Machinery Co., Ltd. Shanghai, China). After ball-milling, the slurry was dried at 80 °C, ground and stored in a vacuum desiccator for further study.

The particle size and zeta potential of the cement powders were measured by dynamic light scattering method using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK, 633 nm He-Ne laser beam with a fixed scattering angle of 90°).

Reactive liquids added to the cement powders for this study were 0.25 M Na_2HPO_4 solution and γ-PGA/Sr aqueous solution. To prepare the γ-PGA/Sr solution, γ-PGA (H^+) was firstly mixed with $SrCO_3$ at a molar ratio of –COOH: Sr^{2+} = 2:1 and then added deionized (DI) water to prepare ≈10 wt% γ-PGA/Sr aqueous solution at room temperature for 4 h and then stored at 4 °C for further study. For evaluating the handling, mechanical and cytocompatibility properties of cement, the cement pastes were prepared by mixing the solid content of CPC, γ-PGA-CPC and Sr-γ-PGA-CPC with corresponding reactive liquids at a liquid-to-solid ratio (L/S) was 0.45 mL/g.

2.3. Injectability, setting times and cohesion properties

The injectability of the cement pastes were tested by the extrusion method using a standard 1 mL syringe with an orifice of 2 mm in inner diameter, as described in a previous study [30]. The cement paste was prepared by mixing the cement powders and reactive liquids and then immediately packed into the syringe. The paste was extruded from the syringe tip by applying a force to the plunger at a speed of 10 mm/min and the extrusion was terminated when the force reached 150 N. Injectability was calculated by the equation,

$$I = [(M_0 - M)/M_0] * 100\% \quad (1)$$

where M_0 is the initial mass of cement loaded into the syringe and M the remaining cement mass in the syringe after extrusion. Each type of cement was tested for three times.

Setting times of cement pastes were tested by the Gillmore method which uses a light needle (113.4 g in weight and 2.12 mm in diameter) and a heavy needle (453.6 g in weight and 1.06 mm in diameter) according to the ASTM C266-04 standard [31]. In brief, cement paste was packed into a cylindrical mold and flatted with a spatula. The initial setting time was determined when the light needle could insert no >1.5 mm from the top surface of cement. The final setting time was determined once the heavy needle could not mark the cement surface with a complete circular dent. Each cement formulation was tested for three times.

The cohesion property in aqueous environment is an indicator of anti-collapsibility of cement. The cohesion property of the cement pastes was evaluated qualitatively. To summarize, the cement paste formed after the mixing powders and reactive liquid was immediately transferred to a 1 mL syringe and then directly injected into DI water. All cement bars were left for hardening in the aqueous solution for

Table 1
Solid and liquid compositions of different cements and Zeta potential of the cement powders.

Type of cement	Solid phases				Zeta potential	Liquid phase
	TCP (wt%)	DCPD (wt%)	γ-PGA (wt%)	HA (wt%)		
CPC	90	10	0	0	−12.3 ± 0.31	0.25 M Na_2HPO_4
γ-PGA-CPC	79.6	8.8	8.9	2.7	−18.4 ± 0.27	0.25 M Na_2HPO_4
Sr-γ-PGA-CPC	79.6	8.8	8.9	2.7	−20.2 ± 0.24	10 wt% γ-PGA/$SrCO_3$